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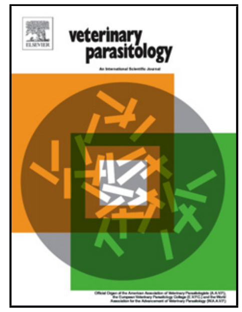

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Author: M.P. Cornelius C. Jacobson R. Dobson R.B. Besier

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**Computer modelling of anthelmintic resistance and worm control outcomes for refugia-based nematode control strategies in Merino ewes in Western Australia**

M.P. Cornelius[a,b*] meghan.cornelius@agric.wa.gov.au, C. Jacobson[a], R. Dobson[a], R.B. Besier[c]

[a]School of Veterinary and Life Sciences, Murdoch University, WA 6150, Australia.

[b]Department of Agriculture and Food Western Australia, Katanning, WA 6317, Australia

[c]Department of Agriculture and Food Western Australia, Albany, WA 6330, Australia.

[*]Corresponding author. Tel.: +61477748430.

**Highlights**

- Leaving a  proportion of adult sheep untreated is an effective TST strategy
- Drenching in autumn rather than summer effectively delayed anthelmintic resistance
- Fully effective anthelmintics are important for worm control and to delay resistance
- More refugia is required to delay resistance when less-effective drugs are used

**Abstract**

This study utilised computer simulation modelling (Risk Management Model for Nematodes) to investigate the impact of different parasite refugia scenarios on the development of anthelmintic resistance and worm control effectiveness. The simulations were conducted for adult ewe flocks in a Mediterranean climatic region over a 20 year time period. Factors explored in the simulation exercise were environment (different weather conditions), drug efficacy, the percentage of the flock left untreated, the timing of anthelmintic treatments, the initial worm egg count, and the number of drenches per annum. The model was run with variable proportions of the flock untreated (0, 10, 20, 30, 40 and 50%), with ewes selected at random so that reductions in the mean worm burden or egg count were proportional to the treated section of the flock. Treatments to ewes were given either in summer (December; low refugia potential, hence highly selective) or autumn (March; less selective due to a greater refugia potential), and the use of different anthelmintics was simulated to indicate the difference between active ingredients of different efficacy. Each model scenario was run for two environments, specifically a lower rainfall area (more selective) and a higher rainfall area (less selective) within a Mediterranean climatic zone, characterised by hot, dry summers and cool, wet winters. Univariate general linear models with least square difference post-hoc tests were used to examine differences between means of factors. The results confirmed that leaving a proportion of sheep in a flock untreated was effective in delaying the development of anthelmintic resistance, with as low as 10% of a flock untreated sufficient to significantly delay resistance, although this strategy was associated with a small reduction in worm control. Administering anthelmintics in autumn rather than summer was also effective in delaying the development of anthelmintic resistance in the lower rainfall environment where all sheep were treated, although the effect of treatment timing on worm control effectiveness varied between the environments and the proportion of ewes left untreated. The use of anthelmintics with higher efficacy delayed the development of resistance, but the initial worm egg count or number of annual treatments had no effect on either the time to resistance development or worm control effectiveness. In conclusion, the modelling study suggests that leaving a small proportion of ewes untreated, or changing the time of treatment, can delay the onset of anthelmintic resistance in a highly selective environment.

**Keywords:** Targeted Selective Treatment; Sheep; Nematodes; Refugia

**Introduction**

The widespread and increasing severity of anthelmintic resistance is considered the greatest threat to efficient sheep nematode (worm) control on a global basis (Kaplan and Vidyashankar, 2012). It is therefore critical to maintain the effectiveness of the current anthelmintic classes that are still effective, and that of any new anthelmintics that may be released (Kaminsky et al., 2008; Little et al., 2011; Leathwick and Besier, 2014). A major factor contributing to the development of anthelmintic resistance in livestock nematodes is the practice of treating all animals in the flock in situations with little refugia, i.e. parasites not exposed to anthelmintics (Besier and Love, 2003; Leathwick et al., 2009). Subsequent to whole flock treatments, the only eggs shed onto pasture are from worms that survived treatment and where there is no refugia as free-living stages on pasture, rapid selection for resistance is likely (van Wyk, 2001; Kaplan, 2010).

Investigations into sustainable parasite control have included “refugia-based” management strategies, which aim to minimise the development of resistance by ensuring the survival of sufficient nematodes with susceptible genotypes to dilute resistant individuals surviving anthelmintic treatment (Van Wyk, 2001; Kenyon et al., 2009; Leathwick and Besier, 2014). In the Mediterranean-environment region of southern Western Australia, where *Trichostrongylus* spp. and *Teladorsagia circumcincta* are the predominant gastrointestinal nematodes, strategic anthelmintic treatments are commonly used in the hot and dry summer period where they impose heavy selection for anthelmintic resistance (Besier and Love, 2003). Two main refugia-based approaches to worm control have been investigated for this environment, both specifically intended for use in mature sheep (Besier, 2012). These involve either a whole-flock treatment to all sheep in autumn (when conditions for survival of free living stages are more favourable than in summer) in order to avoid the heavy resistance selection pressure of summer treatments (Woodgate and Besier, 2010), or a "targeted selective treatment" (TST) approach by which a proportion of a flock is left untreated in summer to retain non-selected (i.e. not anthelmintic exposed) worms in the population (Besier et al., 2010). Both approaches have implications for the likely acceptability to sheep farmers, due to concerns partly over the practicality of implementation, but especially for the effectiveness of parasite control of strategies which require the retention of worm populations in refugia. To achieve wide uptake, it is necessary to demonstrate that the recommended strategies provide a substantial benefit regarding the reduction in the development of anthelmintic resistance, but without a significant loss of worm control efficacy.

Field investigations in Western Australia have confirmed that a TST strategy based on the selection of ewes for treatment (or otherwise) using body condition score is a practical procedure and does not jeopardise wool production when applied in adult sheep flocks in this environment (Besier et al., 2010), or adversely affect body weight or condition score (Cornelius et al., 2014; Cornelius et al., 2015b). However, field studies to determine the longer-term effects on the development of anthelmintic resistance and impacts on worm control effectiveness in the flock into the future are difficult and expensive to conduct in real time. This study utilised computer simulation modelling to investigate the impact of different refugia scenarios on anthelmintic resistance and worm control effectiveness outcomes in adult ewe flocks in a Mediterranean climatic region over a 20 year time period. The modelled scenarios allow comparisons between timing of treatment (summer or autumn), different proportions of a flock left untreated, different locations within the region, and for anthelmintics with different resistance status. The aim of this study was to describe effects of refugia-based nematode control methods on worm control effectiveness and development of anthelmintic resistance, with the goal of developing appropriate worm control programs for a range of sheep management situations and environments that achieve the objectives of both efficiency and sustainability.

## Materials and methods

### *Model – Risk Management Model for Nematodes (RMMN)*

The model, previously described by Dobson et al. (2011a,b) was initially developed in FORTRAN for a DOS computing platform and more recently translated (Dobson, pers. com) to use in Excel under a Windows operating system (Microsoft, 2015).

### *Simulated sheep management*

The model assumptions were that ewes were grazed at a stocking rate of 12 dry sheep equivalent per hectare (DSE: unit of measure to compare feed requirements or farm carrying capacity based on a two-year-old, 45kg Merino wether or non-pregnant/lactating ewe). Ewes rotated between two paddocks each year, changing paddocks in December and back again in April. Lambs were born in June and ran with the ewes until weaned into a separate paddock in October. This cycle continued annually for 20 years. Periods of stress that can diminish the immune response of ewes to worms were taken into account in the model, including that of the peri-parturient relaxation of immunity during early lactation following lambing. Anthelmintic treatments were given to ewes as specified. Lamb treatments were always given at weaning (October) and in summer (December) with the same types of drenches that were used for the ewes in that year. Anthelmintic resistance and worm control effectiveness were assessed across worm populations in the entire flock (ewes and progeny), including the contribution of the lambs through cycling worm populations that originated in the ewes before and following treatment.

Output from the model included two measures: years to anthelmintic resistance and mean worm control effectiveness (as a percentage). Dobson et al. (2011b) provides a full description of how these measures are estimated and how deaths from concurrent nematode infections were estimated, based on model predictions of adult worm burdens. ‘Years to anthelmintic resistance’ indicates the time taken for the resistance (R) allele gene frequency to reach 50% in the infective larval population on pasture. ‘Worm control effectiveness’ is a similar concept to drug “efficacy” with higher effectiveness score representing better control, measuring the weighted average reduction in worm burden, egg counts, production penalty and deaths from an untreated control group over a number of years (Dobson et al., 2011b).

*Factors simulated*

Six factors were explored in the simulation exercise (Table 1): percentage of the flock left untreated, timing of anthelmintic treatments, number of drenches per annum, anthelmintic drugs used, environment (different weather conditions) and initial ewe faecal worm egg count (WEC). This gave rise to 480 separate 20-year simulations.

*Proportion of flock left untreated*: The model was run with variable proportions of the flock untreated (0, 10, 20, 30, 40 and 50%; Table 1), with ewes selected at random so that potential reductions in the mean worm burden or egg count were proportional to the treated percentage of the flock. This refugia strategy aims to allow unselected adult worm populations in sheep to contaminate pasture.

*Time of treatment*: To simulate the strategy of changing the time of strategic anthelmintic treatment of ewes from a highly selective to a less selective time of year, the model was run with treatments to ewes either in summer (December; low refugia potential i.e. few if any infective larvae on pasture, hence highly selective) or autumn (March; less selective due to a greater refugia potential) (Table 1 and Table 2). Lambs were treated to a standard program regardless of ewe treatments, with a treatment at weaning (October) and then in summer (December).

*Treatment frequency:* This was simulated using 1, 2 or 3 treatments to ewes per annum (Table 2).

*Anthelmintic drug choice:* The use of different anthelmintics was simulated to indicate the difference between active ingredients with different efficacy and persistence (Table 1). Treatments included Drug "A", representing a drug at 97% initial efficacy with persistence of 32 days (*T. circumcincta*) and 5 days (*Trichostrongylus* spp.), and initial 3% frequency of resistance allele (representing initial level of drug resistance in the worm population). Drug "B" represents a drug at "100% efficacy" (in practice, 99.999% effective), with no persistence and very low (0.0001%) initial resistance allele frequency. The properties of the Drugs A and B were chosen to represent the persistence properties and expected anthelmintic resistance gene prevalence in the parasite population for two treatment options commonly used in this environment. Different treatment frequencies (frequency of treatments/annum) and rotations of drugs were also simulated. Where one treatment annually was given, this was with either Drug A or Drug B. For two treatments annually, simulations included Drug A only (A-A), Drug B only (B-B), or Drug A and Drug B in rotation (A-B and B-A simulated separately). For three treatments annually, simulations included Drug A only (A-A-A), Drug B only (B-B-B), or Drug A and Drug B in rotation (A-B-A and B-A-B simulated separately). For each year/location result, an average was taken of the means of each possible order of rotation. In each case, the lambs received the same treatment/rotation as the ewes.

*Environments:* The two environments simulated represent a higher rainfall area (Albany) and a lower rainfall area (Kojonup), to cover variation within the Mediterranean climatic conditions (characterised by hot, dry summers and cool, wet winters) typical of Western Australian sheep producing regions. For Albany (high rainfall area), rainfall is approximately 927 mm/annum with the majority falling between the months of May to September, with average annual temperatures of 11.7 (minimum) and 19.5 (maximum) and an annual pasture growing season of approximately 7.5 months duration. For Kojonup (lower rainfall region), rainfall was approximately 526 mm/annum which typically falls entirely in the winter months of June to August, with average annual temperatures of 9.3 (minimum) and 21.4 (maximum), and a 5.5 month growing season. Annual pastures are the predominant pasture systems in this region and the extended dry periods over summer-autumn result in typically zero pasture growth over this period.

*Initial WEC counts*: The initial (starting) mean WEC for ewes in December of the first year of each simulation were set at either 100 eggs per gram (epg) or 500 epg and simulated separately. The mean initial lamb WEC was set at 10% of the ewes' count. Worm species modelled were *Trichostrongylus colubriformis* and *T. circumcincta*. The L3 establishment rate is a measure of sheep immunity to nematodes in young or susceptible animals. The initial establishment rate of infective larvae was set at 1% for ewes to reflect a strong immune response, and at 99% for lambs in which little immunity had developed.

*Statistical analysis*

The data from the model were analysed using SPSS Standard Statistics Version 22.0 (IBM Corporation, Armonk NY). Univariate general linear models with least square difference post-hoc tests were used to examine differences between means of factors. Initial high and low WEC were not significantly different to each other, neither were number of drenches per annum, and therefore were pooled (not separated as different variables) to provide enough replicates to enable statistical differences between the other factors to be tested.

## Results

*Refugia strategies and development of anthelmintic resistance*

The effect of environment, varying the proportion (%) of the flock untreated and timing of anthelmintic treatment on time (years) to the development of anthelmintic resistance are shown in Table 3.

Leaving a proportion of ewes untreated was effective in increasing the time to anthelmintic resistance in the lower rainfall environment (for both summer and autumn treatments) and in the higher rainfall environment (for summer treatment only) (Table 3). Specifically, for summer treatments in the lower rainfall environment (Kojonup), anthelmintic resistance developed approximately 6-9 years earlier in the 0% untreated flock than for all other flocks ($P<0.001$), and 2-3 years earlier in the 10% untreated flock compared to the 30% ($P=0.008$), 40% ($P=0.002$) and 50% ($P=0.002$) flocks. Similarly, for summer treatments in the higher rainfall environment (Albany), anthelmintic resistance developed approximately 2-4 years earlier in the 0% untreated flock than for all other flocks ($P<0.01$) (Table 3). For Autumn treatments in the lower rainfall environment (Kojonup), anthelmintic resistance developed approximately 3-6 years earlier in the 0% untreated flock than for all other groups ($P<0.001$), and approximately 2 years in the 10% untreated flock compared to the 40% ($P=0.031$) and 50% ($P=0.009$) untreated flocks. In contrast, the time to anthelmintic resistance did not differ significantly with different proportions of the flock left untreated for autumn treatments in the higher rainfall environment (Albany), with the exception that resistance developed approximately 3 years earlier in the 0% untreated flock than for the 50% untreated flock (P=0.012; Table 3).

Changing the timing of treatment from summer to autumn delayed anthelmintic resistance by approximately 3 years only in the lower rainfall environment (Kojonup) and only where all ewes were treated (0% untreated; Table 3). Where at least 10% ewes were left untreated, there was no difference in time to anthelmintic resistance observed in flocks given summer treatments compared with flocks given autumn treatments. (Table 3)

*Refugia strategies and worm control outcomes*

Increasing the proportion of flock left untreated reduced worm control effectiveness (%) for both summer and autumn treatments (P<0.001; Table 4). Worm control effectiveness was always higher at Kojonup (lower rainfall) than Albany (higher rainfall), for any category of percentage untreated, or either time of treatment.

Moving the timing of treatment from summer to autumn in the lower rainfall environment resulted in small (but significant) decreases in worm control effectiveness only when the proportion of flock left untreated was 0-10%. In contrast, moving the timing of treatment from summer to autumn in the higher rainfall environment resulted in small (but mostly significant) increases in worm control effectiveness when 10-50% of the flock was untreated (Table 4).

*Anthelmintic drug choice and anthelmintic resistance*

The effect of environment, varying proportion (%) of flock untreated and anthelmintic drug choice on time (years) to development of anthelmintic resistance are shown in Table 5.

The use of the less effective drug (drug A) resulted in anthelmintic resistance sooner than where the highly effective drug (drug B) or the combination of both (A/B rotation) were used in both environments (Table 5). All three of the drug options used were significantly different to each other for all of the proportions of flock left untreated in both environments.

Increasing the proportion of flock untreated from 0% to 10% increased the time to development of anthelmintic resistance in both environments regardless of anthelmintic drug used ($P<0.001$; Table 5), but further increases in the percentage untreated increased the time to resistance only for Drug A at in lower rainfall environment, and for A/B rotations in both environments (Table 5).

*Anthelmintic drug choice and worm control outcomes*

The effect of environment, varying proportion (%) of flock untreated and anthelmintic drug treatment choice on worm control outcomes are shown in Table 6. Worm control effectiveness was significantly different between each proportion of the flock untreated for all drug types used, in both environments (Table 6). Increasing the proportion of flock left untreated reduced worm control effectiveness (%) for both drug choices, including drug rotation, in both environments ($P<0.001$; Table 6).

In the lower rainfall environment, worm control effectiveness was mostly not significant with the exception of Drug A being significantly different to A/B rotations where 0% ($P=0.019$), 30% ($P=0.036$) or 40% (P=0.0.012) flock were untreated. There were no significant differences in worm control between drug types or rotation in the high rainfall environment.

*Effect of initial WEC and treatment frequency on worm control and development of anthelmintic resistance*

As previously noted, both the initial WEC and the number of drenches/annum had no significant effect on either the time to resistance development and worm control effectiveness.

**Discussion**

This study demonstrated the effects of variations in local climatic conditions, drug efficacy and treatment schedules on sustainable nematode management in an environment of high resistance-selection potential, and provides confirmation of earlier modelling studies indicating that refugia-based nematode control strategies can delay anthelmintic resistance and be compatible with maintaining effective worm control (Dobson et al., 2011b; Leathwick, 2012; Learmount et al., 2012). Specifically, the study investigated refugia-based alternatives to the "summer drenching" strategy (Anderson, 1972; 1973), which is widely-used throughout winter rainfall regions in Australia where *T. circumcincta* and *Trichostrongylus* spp. are the major sheep nematodes. Summer drenching is now recognised as applying considerable selection pressure for anthelmintic resistance in environments where there is limited over-summer survival of the free-living infective larvae, such as in Mediterranean climates (Besier and Love, 2003; Woodgate and Besier, 2010). The two alternative strategies tested were specifically aimed at adult sheep (particularly ewes), and include either a TST approach for drenches given in summer (i.e. leaving some sheep untreated), or a whole-flock treatment in autumn rather than in summer, as environmental conditions in autumn are more favourable to larval development and hence provide a greater degree of refugia (Besier, pers. com). Although the treatment strategies tested were applied only to adult Merino ewes, the subsequent effects on the worm populations that developed in their lambs (which were treated at the same times, regardless of ewe treatments) were incorporated into the modelling, such that the outcomes reflect effects in the entire sheep flock on a property. The model ran over a 20-year timeframe using weather data records for each location, and compared the effects of the different strategies on the time (years) taken before the development of anthelmintic resistance and the relative worm control effectiveness, based on an estimate developed by Dobson et al. (2011a) incorporating the pathogenic effects on ewes and lambs (separately) of the modelled worm burdens.

The findings support the concept that leaving a proportion of sheep in a flock untreated can be an effective strategy for delaying the development of anthelmintic resistance, and adds to similar observations in different environments (Hoste et al., 2002; Leathwick et al., 2006; Cringoli et al., 2009; Gallidis et al., 2009; Greer et al., 2009; Stafford et al., 2009; Gaba et al., 2010). The findings were consistent with previous field studies in Western Australia that found no change in anthelmintic resistance status when 10% of a lamb flock were left untreated compared with increased anthelmintic resistance where all lambs were treated (Besier, 2001). However, the study by Besier (2001) found that partially-drenched flocks suffered significant parasitism in the following winter, therefore suggesting that effects on both control effectiveness as well as the development of anthelmintic resistance need to be considered when evaluating refugia-based control strategies. More recently, computer modelling studies by Dobson et al. (2011a) indicated that where anthelmintic efficacy is high, a useful level of refugia with a minimal risk to lamb production could be provided by leaving a lower percentage untreated (4-10%). Similarly, field and modelling studies in Western Australia demonstrated that adult ewe flocks with a proportion of ewes left untreated in winter did not result in meaningful loss of weight or body condition (Besier et al., 2010; Cornelius et al., 2014; Cornelius et al., 2015b). The present study investigated implementing refugia-based strategies for adult ewes, which are expected to show a greater tolerance of worm burdens than lambs, and confirmed that leaving as few as 10% of the flock untreated in summer was sufficient to significantly delay the onset of anthelmintic resistance, regardless of the environment or treatment choice. There was an environmental and seasonal effect on the effectiveness of this strategy. In the lower rainfall environment (Kojonup), resistance developed four years (autumn) to six years (summer) later when 10% were left untreated, compared with a drench to the entire ewe flock. Increasing the percentage of ewes untreated resulted in further benefit in resistance delay when up to 30% ewes were untreated, with no additional benefit beyond this. However, in the higher rainfall environment, the benefit in resistance delay only occurred for up to 10% ewes untreated, and the magnitude of benefit (2.5 year delay) was smaller than for the lower rainfall environment. This difference in response to a TST strategy was consistent with the relative favourability for worm larval development and degree of parasite refugia between the two locations. Larval persistence is severely restricted during the prolonged hot and dry summer periods at Kojonup. In contrast, a greater proportion of infective larvae are expected to survive over summer in the more temperate environment and higher rainfall location of Albany, hence providing sufficient refugia for non-resistant worms to obviate the benefit of TST strategies (Besier, 2001; Woodgate and Besier, 2010).

Leaving a proportion of sheep in the flock untreated resulted in a decrease in worm control effectiveness in ewes due to the increased contamination of pastures with worm eggs, which is a potential disadvantage of TST strategies (Leathwick et al., 2006; Leathwick et al.. 2008; Waghorn et al., 2008). For both summer and autumn treatments and in both environments, the modelled worm control effectiveness was reduced for each increment in the proportion of ewes left untreated. However, whether this would result in economically-significant production loss was not quantified and requires further investigation. Besier et al. (2010) showed that where 50% or more of ewes were left untreated in summer, differences in worm control was only significant immediately after treatments were administered, and mean flock egg counts remained low in both TST and whole-flock treated groups from treatment onwards.

A change of drench timing from summer to autumn was also an effective strategy for delaying anthelmintic resistance when all sheep were treated in the lower rainfall environment of Kojonup. This strategy has been shown to be less selective for anthelmintic resistance in environments where there is negligible survival of infective larvae over summer (Woodgate and Besier, 2010), and changing strategic treatments from summer to autumn has been recommended as a sustainable and effective refugia-based worm control strategy in south-west Western Australia. However, the resistance-delay benefit was not observed for the higher rainfall environment in the present study, presumably again reflecting the greater refugia on the pasture for infective larvae in a more temperate environment (compared to Kojonup) over the summer months (Besier, 2001; Besier and Love, 2003). Environmental differences in control effectiveness in response to changing the timing of treatment from summer to autumn were also observed. In the lower rainfall environment, drenching in summer resulted in less effective worm control when more than 10% of the flock was left untreated, demonstrating that the “traditional” summer treatments provided slightly better protection against worms (although with a greater risk that anthelmintic resistance will develop) where more than 90% ewes were treated. A recent survey by Cornelius et al. (2015a) provided evidence of widespread adoption of the “autumn drenching” strategy by farmers in Western Australian regions with only 38% respondents giving a summer drench for ewes in the survey period. The results of the present modelling study support the notion that both this strategy and TST slows the development of anthelmintic resistance in Mediterranean environment, without serious worm control disadvantage in the majority of situations.

As expected, anthelmintic efficacy (treatment choice) had a major effect on the rate of development of anthelmintic resistance. This is consistent with other reports that continued use of anthelmintics to which resistance has already developed can rapidly increase the rate of development of resistance (Leathwick and Besier, 2014), and that this is mediated by environmental effects impacting the degree of refugia. In the present study, resistance developed in less than seven years to a drench that was 97% effective compared to over 16 years for a drug with extremely high efficacy (>99.99%) in the lower refugia (higher resistance-selection) environment of Kojonup. This observation was consistent with previous computer model predications from New Zealand indicating that over 30 times the refugia was required to achieve an equivalent delay in the time for anthelmintic resistance to develop for a drug of 95% efficacy compared to when a fully-effective anthelmintic was used (Leathwick et al., 2009). However, the rate of anthelmintic resistance development to drugs of both levels of efficacy was reduced when a proportion of ewes were left untreated in the present study. The effectiveness of TST for delaying anthelmintic resistance was evident regardless of anthelmintic efficacy, although the magnitude of benefit was greatest when the fully-effective anthelmintic was used, with resistance not observed in either environment in those sets of simulations where at least 10% ewes were left untreated. An environmental interaction with anthelmintic efficacy was also apparent, with a shorter time to resistance for all TST treatment regimes at Kojonup compared to Albany, and with a positive relationship between the increase in time to resistance and increase in proportion of ewes left untreated observed only at Kojonup. At Albany, a drug with 97% efficacy apparently removed sufficient resistant worms to obviate the effect of increasing levels of refugia as the percentage of untreated ewes increased.

Rotations of anthelmintics at intervals has been widely accepted by farmers in Australia, despite indications that this is likely to have only a marginal effect on reducing selection for resistance in comparison to the use of effective anthelmintics (Barnes et al., 1995; van Wyk, 2001). This is consistent with results from the present study, where an annual rotation between drugs with an efficacy of either 97% (and persistent activity) or 99.999% (and no persistence) increased the time to resistance development by only a short period compared to continuous use of the less-effective drug, which suggests that there is little point in rotating two or more anthelmintics where none are fully effective. However, using each drug modelled here continuously until resistance developed, then switching to the other drug, but with 10% of sheep left untreated, would effectively double the useful drench life, for little reduction in worm control effectiveness compared to whole-flock treatment. This result supports the contention that the use of high-efficacy anthelmintics within a refugia strategy is highly sustainable with minimal effect on the efficiency of worm control.

Neither the initial worm egg count of the ewes or the number of drench treatments had a significant effect on resistance development or worm burdens. While it seemed likely that a five-fold difference in the initial worm egg counts of ewes (100 epg vs. 500 epg) would lead to less-effective worm control, particularly when a proportion of the flock were not treated, the hot and dry conditions typical of Mediterranean climates during summer and early autumn would have prevented significant larval development in both environments, regardless of the rate of worm egg deposition. However, it is possible that if the initial pasture contamination rate was higher than in these simulations, this could be a significant factor determining effectiveness of worm control due to the greater level of larval development once conditions became favourable from late autumn onward. Similarly, it may also be expected that the number of anthelmintic treatments given to ewes (between one and three per year) would affect both the development of anthelmintic resistance and the effectiveness of worm control, but no significant effects were found. This appears to be because the additional treatments were given at the time of routine management operations later in the year (pre-lambing in June, or at lamb weaning in October), when pasture conditions were at their most favourable for worm egg development. The number of infective larvae in refugia would be at its seasonal highest at this time, hence minimising the development of resistance, and the use of short-acting drenches would have only a limited effect in reducing worm burdens.

Despite the potential sustainability benefits, conceptual barriers to the adoption of TST strategies by sheep farmers are likely to apply in all locations due to concerns about potential sheep production and worm related disease, but an understanding by farmers of the benefits and level of risk associated with these strategies will aid in their adoption (Cornelius et al., 2015a). Factors affecting the acceptability of TST to farmers have been recognised for some time (Besier, 2012) and ultimately depend on the availability of practical systems for identifying the sheep that are most likely to benefit from treatment, as well as confidence that sheep production will not be adversely affected by the survival of worm populations from untreated ewes. The most practicable TST indicators will need to be quick and simple to apply, easily learnt, and allow treatment decisions to be made on-the-go (Kenyon et al., 2009; Besier, 2012). Recent investigations in Australia demonstrated that body condition score is a simple and practical indicator for a TST strategy in large flocks of adult sheep, and can be used to identify individual ewes at risk of compromised production or welfare if left untreated (Besier et al., 2010; Cornelius et al., 2014). These concluded that in Mediterranean environments, and where ewes are in good body condition, the random selection of well-conditioned ewes is a feasible and effective indicator of the sheep to leave untreated (Cornelius et al., 2015b).

Another more fundamental barrier to adoption of refugia and TST strategies occurs where sheep farmers do not appreciate the potential impact of anthelmintic resistance until severe production loss or clinical disease becomes evident, which may not occur until serious multiple drug resistance develops (van Wyk et al., 2006). This is consistent with findings from a survey conducted in 2012 in Western Australia (Cornelius et al., 2015a) in which one-third of survey respondents did not perceive drench resistance to be an important problem. This was surprising given the high prevalence of resistance in the region, but despite this, more than 65% of respondents were aware of the TST concept, and 25% had implemented it in some form. This suggests that those farmers who perceive drench resistance to be a problem are likely to accept TST as a viable management strategy. Results from the present study provide a good basis for developing optimal refugia-based sustainable control programs that slow the development of anthelmintic resistance while maintaining sheep production and health.

**Conclusion**

This study demonstrated that the refugia-based strategies of either leaving a proportion of ewes untreated in summer (targeted selective treatment), or administering anthelmintic treatment in autumn rather than in summer, were effective in delaying the development of anthelmintic resistance in a Mediterranean climate. Increasing the proportion of sheep left untreated increased the degree of refugia provided, and leaving as low as 10% of a flock untreated was sufficient to significantly delay anthelmintic resistance in an environment where few infective larvae survive for a prolonged period. This study also confirmed the importance of using high-efficacy anthelmintics for both effective worm control and to minimise anthelmintic resistance, and that significantly larger refugia populations are required to delay resistance development when less-effective drugs are used in highly selective environments.

**Conflict of interest statement**

The corresponding author (MC) was employed by the pharmaceutical company Jurox Animal Health from January 2013 to February 2014 but the company had no input or influence on the investigation. The computer model RMMN is owned by Novartis Animal Health (now Elanco Animal Health) but there was no input or influence on the investigation by either company.

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**Tables**

*Table 1. Factors used for multiple model simulations.*

| Classification | Factors | Variables used in simulations |
|---|---|---|
| Refugia factors | % untreated in flock | 0, 10, 20, 30, 40, 50% |
| | Treatment timing | Summer, Autumn |
| Parasite control method factors | Treatment frequency/annum | 1, 2, 3 |
| | Drugs used | Drug A (approximately 97% efficacy, short period of persistence ) |
| | | Drug B (approximately 99.999% efficacy, no persistence) |
| | | Within year rotation of A and B |
| Random factors | Environment (weather) | High rainfall, low rainfall |
| | Initial ewe worm egg count | 100epg, 500epg |

*Table 2. Timing and number of simulated anthelmintic treatments for ewes.*

| Drench timing | Drench/annum | Month drench administered |
|---|---|---|
| Summer | 1 | December |
| | 2 | December, October |
| | 3 | December, June, October |
| Autumn | 1 | March |
| | 2 | March, October |
| | 3 | March, June, October |

*Table 3. Effect of proportion (%) of flock treated and timing of treatment on mean time (years) to anthelmintic resistance in two environments.*

| **Environment** | **% of flock untreated** | **Years to AR*** | | |
|---|---|---|---|---|
| | | **Summer** | **Autumn** | **P Value** |
| Lower rainfall (Kojonup) | 0 | 9.5[c] | 12.8[c] | 0.007 |
| | 10 | 15.8[b] | 16.1[b] | ns |
| | 20 | 16.9[ab] | 17.0[ab] | ns |
| | 30 | 18.1[a] | 17.8[ab] | ns |
| | 40 | 18.5[a] | 18.1[a] | ns |
| | 50 | 18.6[a] | 18.5[a] | ns |
| | **P Value** | <0.001 | <0.001 | |
| High rainfall (Albany) | 0 | 14.0[b] | 14.7[b] | ns |
| | 10 | 16.5[a] | 16.1[ab] | ns |
| | 20 | 16.9[a] | 16.2 [ab] | ns |
| | 30 | 17.1[a] | 16.5 [ab] | ns |
| | 40 | 17.1[a] | 16.5 [ab] | ns |
| | 50 | 17.6[a] | 17.3[a] | ns |
| | **P Value** | 0.004 | ns | |

*AR: anthelmintic resistance. Means are averaged over worm species, drugs, the number of treatments given and initial worm egg counts.

ns: not significant ($P>0.05$)

[abc] For each environment, values in columns with different superscripts are significantly different ($P<0.05$)

*Table 4. Effect of proportion (%) of flock treated and timing of treatment on mean worm control effectiveness (%) in two environments.*

| | | **Worm control effectiveness* (%)** | | |
|---|---|---|---|---|
| **Environment** | **% of flock untreated** | **Summer** | **Autumn** | **P Value** |
| Lower rainfall (Kojonup) | 0 | 86.9$^{a}$ | 85.3$^{a}$ | 0.025 |
| | 10 | 84.6$^{b}$ | 81.8$^{b}$ | <0.001 |
| | 20 | 76.1$^{c}$ | 75.3$^{c}$ | ns |
| | 30 | 68.0$^{d}$ | 68.6$^{d}$ | ns |
| | 40 | 62.3$^{e}$ | 62.6$^{e}$ | ns |
| | 50 | 57.5$^{f}$ | 57.8$^{f}$ | ns |
| | **P Value** | <0.001 | <0.001 | |
| High rainfall (Albany) | 0 | 88.4$^{a}$ | 88.7$^{a}$ | ns |
| | 10 | 85.6$^{b}$ | 87.7$^{b}$ | <0.001 |
| | 20 | 83.9$^{c}$ | 85.4$^{c}$ | 0.001 |
| | 30 | 81.6$^{d}$ | 82.8$^{d}$ | 0.021 |
| | 40 | 78.2$^{e}$ | 79.5$^{e}$ | 0.054 |
| | 50 | 75.7$^{f}$ | 77.7$^{f}$ | 0.001 |
| | **P Value** | <0.001 | <0.001 | |

* weighted average reduction in worm burden, egg counts, production penalty and deaths from an untreated control group over a number of years

ns: not significant (P>0.05)

$^{abcdef}$For each environment, values in columns with different superscripts are significantly different (P<0.05)

*Table 5. Effect of proportion (%) of flock treated and anthelmintic drug choice on mean time (years) to anthelmintic resistance in two environments.*

| **Environment** | **% of flock untreated** | **Years to AR*** | | | | **P Value** |
|---|---|---|---|---|---|---|
| | | **Drug A**[#] | **Drug B**[##] | **A/B Rotations**[^] | | |
| | | | | *Drug A* | *Drug B* | |
| Lower rainfall (Kojonup) | 0% | 6.6$^{f}$ | 16.1$^{b}$ | 8.9$^{e}$ | 10.8$^{e}$ | <0.001 |
| | 10% | 12.5$^{e}$ | 21.0$^{a}$ | 14.2$^{d}$ | 14.9$^{d}$ | <0.001 |
| | 20% | 13.5$^{d}$ | 21.0$^{a}$ | 15.7$^{c}$ | 16.6$^{c}$ | <0.001 |
| | 30% | 14.4$^{c}$ | 21.0$^{a}$ | 17.1$^{b}$ | 18.3$^{b}$ | <0.001 |
| | 40% | 14.8$^{b}$ | 21.0$^{a}$ | 17.8$^{ab}$ | 18.9$^{ab}$ | <0.001 |
| | 50% | 15.1$^{a}$ | 21.0$^{a}$ | 18.0$^{a}$ | 19.2$^{a}$ | <0.001 |
| | **P Value** | <0.001 | <0.001 | <0.001 | <0.001 | |
| High rainfall (Albany) | 0% | 9.7$^{c}$ | 19.9$^{b}$ | 12.0$^{e}$ | 13.7$^{e}$ | <0.001 |
| | 10% | 13.1$^{a}$ | 21.0$^{a}$ | 14.0$^{d}$ | 15.2$^{d}$ | <0.001 |
| | 20% | 13.1$^{a}$ | 21.0$^{a}$ | 14.2$^{cd}$ | 15.8$^{cd}$ | <0.001 |
| | 30% | 13.1$^{a}$ | 21.0$^{a}$ | 14.6$^{bc}$ | 16.4$^{bc}$ | <0.001 |
| | 40% | 13.1$^{a}$ | 21.0$^{a}$ | 14.7$^{ab}$ | 16.5$^{b}$ | <0.001 |
| | 50% | 13.4$^{a}$ | 21.0$^{a}$ | 15.4$^{a}$ | 17.8$^{a}$ | <0.001 |
| | **P Value** | <0.001 | <0.001 | <0.001 | <0.001 | |

*AR: anthelmintic resistance. Means are averaged over worm species, drugs, the number of treatments given and initial worm egg counts. Where time to anthelmintic resistance is shown as 21 years, resistance did not occur in that set of simulations.

[#] Drug A 97% effective, some persistence

[##]Drug B: 99.999% effective, no persistence

^Years to AR for drug rotations is an average for all possible orders of drug rotations in each year, ie, A/B and B/A.

[abcd] For each environment, values in columns with different superscripts are significantly different (P<0.05)

*Table 6. Effect of proportion (%) of flock treated and anthelmintic drug choice on mean worm control effectiveness (%) in two environments.*

| **Environment** | **% of flock untreated** | **Drugs used** | | | **P Value** |
|---|---|---|---|---|---|
| | | **Drug A**[#] | **Drug B**[##] | **A/B Rotations**[^] | |
| Lower rainfall (Kojonup) | 0% | 85.0[a*] | 85.8[a] | 87.0[a*] | ns |
| | 10% | 82.3[b] | 83.2[b] | 83.9[b] | ns |
| | 20% | 75.2[c] | 75.0[c] | 76.5[c] | ns |
| | 30% | 66.9[d*] | 68.5[d] | 69.2[d*] | ns |
| | 40% | 60.6[e*] | 62.5[e] | 63.7[e*] | 0.040 |
| | 50% | 56.0[f] | 58.7[f] | 58.1[f] | ns |
| | **P Value** | <0.001 | <0.001 | <0.001 | |
| High rainfall (Albany) | 0% | 88.1[a] | 88.7[a] | 88.8[a] | ns |
| | 10% | 86.4[b] | 86.3[b] | 87.1[b] | ns |
| | 20% | 84.6[c] | 84.0[c] | 85.2[c] | ns |
| | 30% | 82.1[d] | 81.7[d] | 82.7[d] | ns |
| | 40% | 78.9[e] | 78.8[e] | 78.8[d] | ns |
| | 50% | 76.4[f] | 76.1[f] | 77.3[f] | ns |
| | **P Value** | <0.001 | <0.001 | <0.001 | |

ns: non-significant (P>0.05)

[abcd] For each environment, values in columns with different superscripts are significantly different (P<0.05)

*For each environment, values in rows (different drug choice) with * are significantly different

[#] Drug A 97% effective, some persistence

[##]Drug B: 99.999% effective, no persistence

^Worm control effectiveness for A/B rotations is an average for all possible orders of drug rotations in each year, ie, A/B and B/A.